

Preparing DNA for transformation

We will be starting with DNA straight from the parts registry. It is given to us as dried DNA.

1. Locate the correct well on the kit plate for the plasmid you are trying to transform. (We will go through this together).
2. Pipette up 10ul of dH₂O and, using the tip to puncture the foil, drop it into the correct well. Pipette up and down to resuspend, then let sit 5 minutes and repeat.
3. Transfer the solution to a properly labeled tube.

Transformation into cells

Competent stocks are kept in the -80C freezer (upstairs). Today, we are using chemically competent cells.

4. While preparing the DNA, thaw an aliquot of cells **on ice**.
5. We are only using 25ul of cells per transformation, so split cells into 25ul aliquots once thawed (keep on ice).
6. To each tube of cells, add 5ul of desired resuspended DNA, making sure to clearly mark the tube. Do **not** pipette up and down to mix.
7. Let sit on ice for 30 minutes. Meanwhile, heat water bath up to 42C.
8. Heat shock cells at 42C without shaking for 30 seconds.
9. Let sit on ice for 2 minutes.
10. Add 200ul of prewarmed LB to each tube, then let recover for 1 hour on a shaking incubator at 37C.
11. Plate mixture onto a selective plate with the appropriate antibiotic. Incubate upside down at 37C overnight.